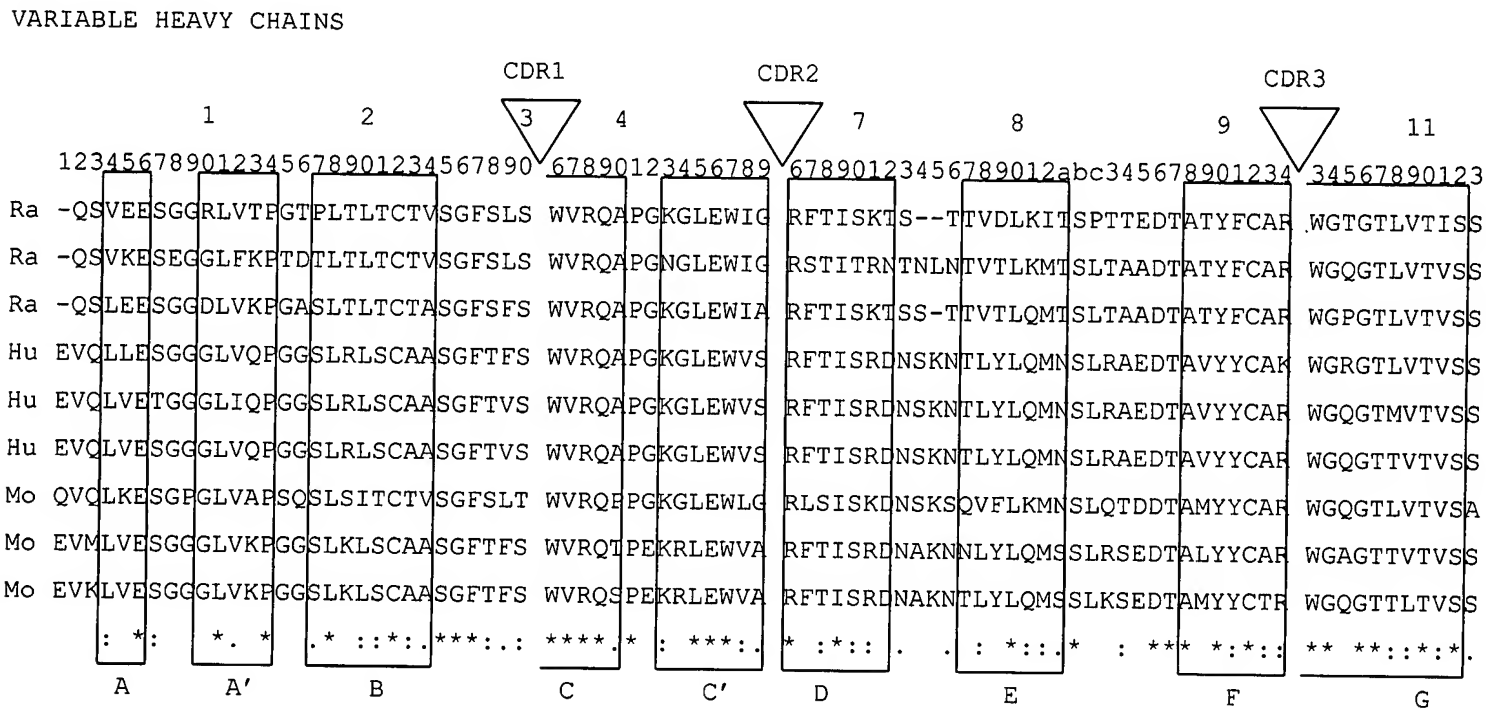
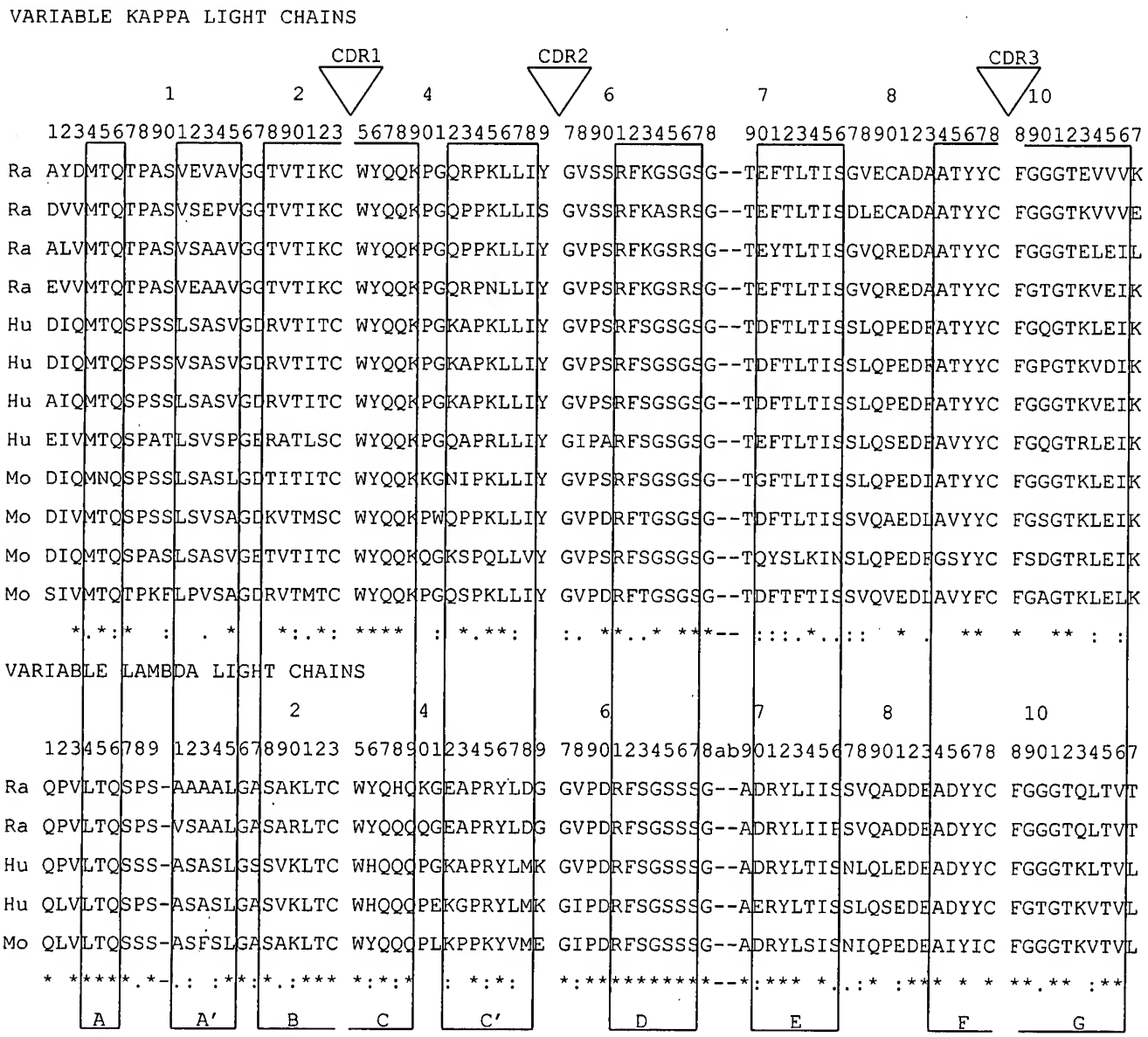


Multiple sequence alignment of rabbit, human and murine VH frameworks

FIG. 1A



Multiple sequence aligment of rabbit, human and murine VL frameworks **FIG. 1B**

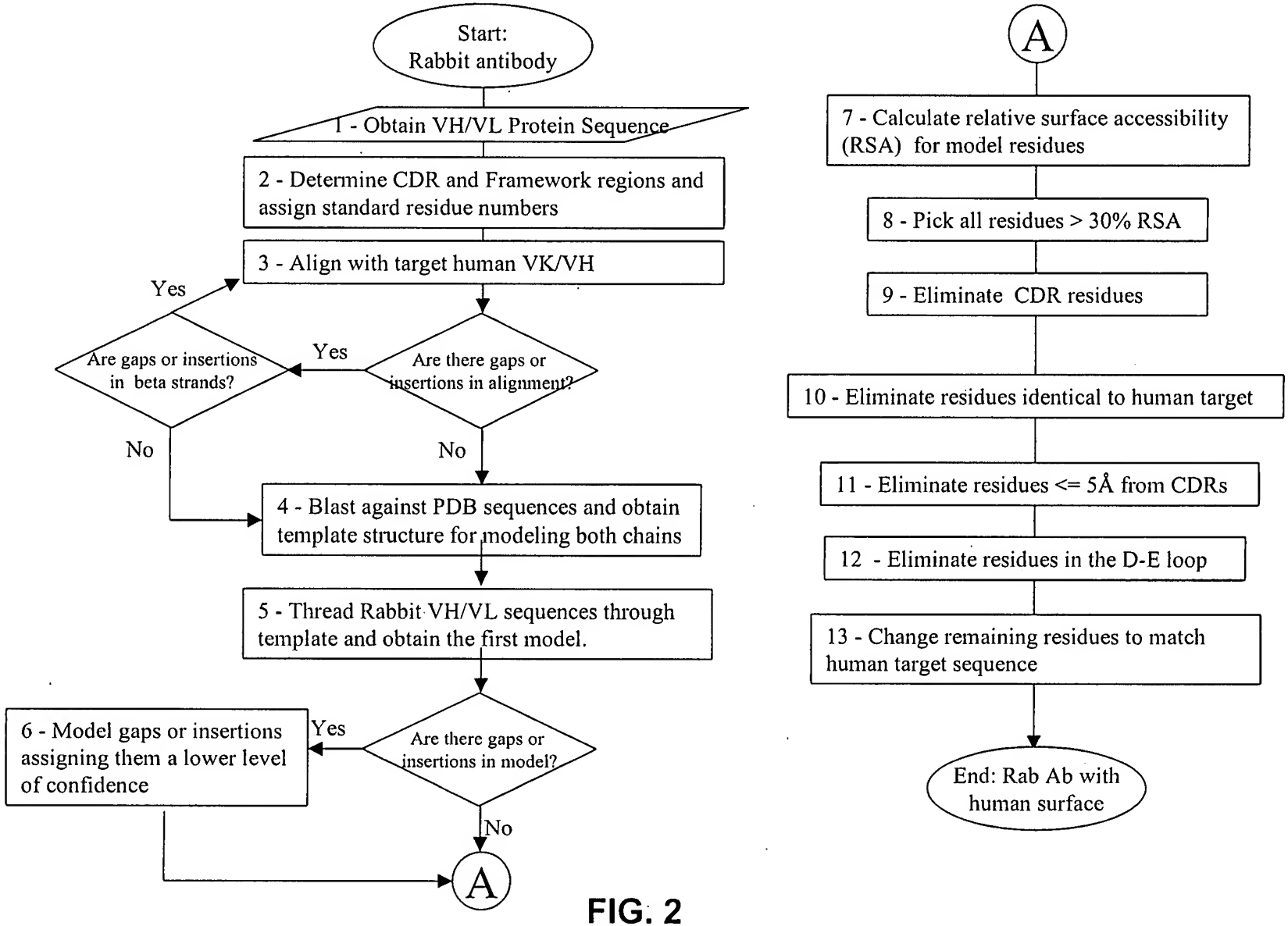

Start:
Rabbit antibody
1 - Obtain VH/VL Protein Sequence
2 - Determine CDR and Framework regions and assign standard residue numbers
3 - Align with target human VK/VH
Are there gaps or insertions in alignment?
Yes
Are gaps or insertions in beta strands?
Yes
No
No
4 - Blast against PDB sequences and obtain template structure for modeling both chains
5 - Thread Rabbit VH/VL sequences through template and obtain the first model.
Are there gaps or insertions in model?
Yes
6 - Model gaps or insertions assigning them a lower level of confidence
No
A
A
7 - Calculate relative surface accessibility (RSA) for model residues
8 - Pick all residues > 30% RSA
9 - Eliminate CDR residues
10 - Eliminate residues identical to human target
11 - Eliminate residues <= 5Å from CDRs
12 - Eliminate residues in the D-E loop
13 - Change remaining residues to match human target sequence
End: Rab Ab with human surface

FIG. 2

FRAMEWORK 1 RELATIVE SOLVENT ACCESSIBILITY

	VARIABLE KAPPA										V. LAMBDA			
	mouse 12E8		mouse 6FAB		mouse 2FBJ		mouse 1A2Y		rabbit B1mdl		human 2FB4		human 8FAB	
1	D	**54**	D	**54**	E	**73.**	D	**46.**	D	**42**	Q	**60.**		
2	I	7	I	4	I	6	I	13	I	9	S	**45**		
3	V	**32**	Q	**38**	V	**37**	V	**33**	V	**31**	V	30	E	**43.**
4	M	5	M	7	L	9	L	7	M	7	L	0	L	6
5	T	27	T	18	T	**33**	T	**38**	T	27	T	29	T	**39**
6	Q	4	Q	7	Q	8	Q	10	Q	8	Q	4	Q	8
7	S	**38**	I	**34**	S	23	S	27	T	**35**	P	21	P	18
8	Q	**34**	P	18	P	25	P	18	P	21	P	**44**	P	**44**
9	K	**37**	S	**37**	A	**32**	A	**38**	S	**40**	S	**37**	S	25
10	F	28	S	29	I	**55**	S	**31**	S	25				
11	M	17	L	17	T	13	L	14	V	13	A	6	V	15
12	S	26	S	**32**	A	22	S	**37**	S	26	S	30	S	19
13	T	1	A	5	A	0	A	1	A	12	G	2	V	4
14	S	16	S	23	S	17	S	17	A	28	T	26	S	20
15	V	**36**	L	**36**	L	**41**	V	**34**	V	**47**	P	**37**	P	**38**
16	G	23	G	22	G	24	G	26	G	21	G	28	G	**32**
17	D	21	D	25	Q	26	E	26	G	12	Q	**30**	Q	**36**
18	R	27	R	**48**	K	**46**	T	**34**	T	**41**	R	**52**	T	26
19	V	2	V	3	V	6	V	7	V	12	V	4	A	1
20	S	21	S	27	T	**31**	T	28	T	29	T	28	R	**46**
21	I	1	I	0	I	2	I	3	I	2	I	1	I	1
22	T	29	S	20	T	23	T	21	K	**37**	S	28	T	25
23	C	3	C	0	C	0	C	0	C	1	C	1	C	0

	VARIABLE HEAVY							
	mouse 12E8		mouse 6FAB		mouse 2FBJ		rabbit B1mdl	
1	E	**73**	E	**52**	E	**77**		
2	V	23	V	8	V	20	Q	**31**
3	Q	**30**	Q	**32**	K	**38**	S	**35**
4	L	3	L	4	L	6	L	6
5	Q	**35**	Q	**35**	L	**38**	E	25
6	Q	4	Q	4	E	7	E	0
7	S	14	S	27	S	27	S	17
8	G	26	G	13	G	25	G	20
9	A	**35**	V	28	G	20	G	15
10	E	24	E	24	G	13	G	25
11	V	**33**	L	**36**	L	29	L	**47**
12	V	7	V	7	V	7	V	9
13	R	**46**	R	**45**	Q	**44**	K	**44**
14	S	19	A	23	P	**31**	P	27
15	G	**33**	G	26	G	28	G	28
16	A	11	S	18	G	10	A	15
17	S	**35**	S	21	S	**35**	S	26
18	V	7	V	3	L	9	L	14
19	K	**36**	K	**33**	K	**42**	A	29
20	L	0	M	0	L	0	L	0
21	S	13	S	12	S	17	T	24
22	C	1	C	0	C	0	C	0
23	T	21	K	**31**	A	26	K	**40**
24	A	6	A	5	A	6	A	1
25	S	25	S	20	S	21	S	29
26	G	**39**	G	**33**	G	30	G	**37**
27	F	9	Y	13	F	10	F	13
28	N	**39**	T	**38**	D	29	S	**31**
29	I	1	F	5	F	0	F	3
30	K	**32**	T	22	S	22	S	23

FIG. 3A

FRAMEWORK 2 RELATIVE SOLVENT ACCESSIBILITY																						
	VARIABLE KAPPA										V. LAMBDA				VARIABLE HEAVY							
	mouse 12E8		mouse 6FAB		mouse 2FBJ		mouse 1A2Y		rabbit B1mdl		human 2FB4		human 8FAB			mouse 12E8		mouse 6FAB		mouse 2FBJ		rabbit B1mdl	
35	W	0	W	0	W	0	W	0	W	1	W	1	W	0	**36**	W	0	W	1	W	0	W	3
36	Y	0	Y	0	Y	0	Y	0	Y	1	Y	0	Y	0	**37**	V	0	V	0	V	0	V	0
37	Q	8	Q	5	Q	3	Q	12	Q	11	Q	13	Q	11	**38**	K	3	K	7	R	8	R	3
38	Q	9	Q	6	Q	6	Q	8	Q	7	Q	1	Q	7	**39**	Q	16	Q	8	Q	8	Q	8
39	K	22	K	25	K	26	K	29	K	21	L	**31**	K	16	**40**	R	16	R	**30**	A	10	A	19
40	P	**50**	P	30	S	**44**	Q	**61**	P	**51**	P	**36**	P	**47**	41	P	**35**	P	**36**	P	**42**	P	**49**
41	G	**38**	D	**59**	G	**43**	G	**40**	G	**44**	G	18	G	**47**	42	E	**62**	G	**37**	G	**48**	G	**39**
42	Q	**31**	G	12	T	25	K	**48**	Q	**41**	M	**34**	R	**38**	**43**	K	**39**	Q	**38**	K	**43**	K	23
43	S	12	T	**35**	S	7	S	9	P	20	A	23	A	15	**44**	G	13	G	17	G	13	G	16
44	P	2	I	7	P	2	P	0	P	7	P	5	P	4	**45**	L	7	L	6	L	6	L	13
45	K	**32**	K	**33**	K	**34**	Q	**40**	K	**48**	K	**44**	V	26	**46**	E	15	E	23	E	22	E	29
46	L	4	L	2	P	13	L	13	L	9	L	8	M	5	**47**	W	5	W	2	W	3	W	5
47	M	3	L	2	W	5	L	3	L	4	L	2	V	0	**48**	I	0	I	0	I	0	I	2
48	I	0	I	0	I	0	V	0	I	0	I	0	I	0	**49**	G	0	G	0	G	0	A	0
49	Y	16	Y	13	Y	20	Y	11	Y	27	Y	17	Y	11									

FIG. 3B

FRAMEWORK 3 RELATIVE SOLVENT ACCESSIBILITY																										
	VARIABLE KAPPA										V. LAMBDA				VARIABLE HEAVY											
	mouse 12E8		mouse 6FAB		mouse 2FBJ		mouse 1A2Y		rabbit B1mdl		human 2FB4		human 8FAB			mouse 12E8		mouse 6FAB		mouse 2FBJ		rabbit B1mdl				
57	G	**40**	G	**35**	G	**38**	G	**44**	G	**38**	G	**43**	G	**41**												
58	V	8	V	10	V	11	V	13	V	9	V	7	I	11												
59	P	21	P	21	P	14	P	24	P	24	P	22	P	19												
60	D	**44**	S	**50**	A	**48**	S	**51**	S	**50**	D	**50**	Q	**58**												
61	R	14	R	12	R	15	R	16	R	17	R	14	R	11	**66**	K	15	K	19	K	20	R	10			
62	F	1	F	1	F	2	F	3	F	2	F	4	F	3	**67**	A	4	T	2	F	0	F	3			
63	T	21	S	25	S	22	S	25	S	21	S	25	S	26	**68**	T	26	T	24	I	**36**	T	27			
64	G	6	G	6	G	8	G	6	G	4	G	12	S	7	**69**	M	4	L	1	I	1	I	5			
65	S	27	S	29	S	**31**	S	26	S	29	S	**34**	S	27	**70**	T	20	T	23	S	20	S	18			
66	G	20	G	15	G	15	G	21	G	22	K	27	T	**32**	**71**	A	20	V	15	R	7	K	16			
67	S	18	S	**39**	S	**40**	S	**33**	Y	**48**	S	**40**	S	**37**	**72**	D	26	D	21	D	29	T	**35**			
68	G	10	G	10	G	16	G	12	G	2	G	28	G	28	73	T	25	K	**38**	N	12					
															74	S	**54**	S	**50**	A	**46**	S	19			
															75	S	24	S	20	K	**46**	A	26			
69	T	19	T	18	T	24	T	24	T	15	A	14	T	22	76	N	9	S	15	N	17	T	**32**			
70	D	**32**	D	30	S	**31**	Q	**37**	E	**34**	S	25	T	13	**77**	T	4	T	3	S	4	T	19			
71	F	0	Y	1	Y	3	Y	2	F	0	A	2	V	1	**78**	A	1	A	2	L	0	V	4			
72	T	14	S	19	S	14	S	16	T	7	S	16	T	9	**79**	Y	22	Y	17	Y	13	T	19			
73	L	1	L	1	L	1	L	1	L	0	L	0	L	0	**80**	L	0	M	0	L	0	L	1			
74	T	4	T	10	T	9	K	28	T	14	A	4	T	12	**81**	Q	26	Q	27	Q	24	Q	22			
75	I	0	I	0	I	2	I	1	I	2	I	1	I	0	**82**	L	2	L	2	M	0	M	0			
76	S	18	S	30	N	**31**	N	22	S	17	G	24	S	28	**82a**	S	20	R	**31**	S	14	T	16			
77	N	**34**	N	25	T	23	S	30	D	**38**	G	6	G	19	82b	S	**37**	S	27	K	**49**	T	28			
78	M	0	L	2	M	0	L	0	L	11	L	2	V	1	82c	L	2	L	1	V	0	L	2			
79	Q	23	E	25	E	27	Q	27	E	24	Q	**34**	Q	23	83	T	24	T	18	R	**42**	T	21			
80	S	**37**	Q	26	A	10	P	**39**	C	**42**	S	**36**	A	**38**	84	S	**32**	S	**38**	S	**32**	A	**34**			
81	E	**41**	E	**36**	E	**35**	E	**36**	A	18	E	**42**	E	21	85	E	**36**	E	**35**	E	**45**	A	24			
82	D	0	D	0	D	2	D	1	D	1	D	1	D	2	86	D	3	D	4	D	2	D	0			
83	L	19	I	14	A	5	F	10	A	22	E	24	E	18	87	T	12	S	11	T	12	T	11			
84	A	3	A	2	A	2	G	8	A	2	T	6	A	0	**88**	A	1	A	2	A	1	A	1			
85	D	20	T	13	I	21	S	12	T	5	D	5	D	17	**89**	V	15	V	15	L	23	T	15			
86	Y	0	Y	0	Y	0	Y	0	Y	1	Y	0	Y	0	**90**	Y	1	Y	1	Y	0	Y	0			
87	F	2	F	1	Y	3	Y	4	Y	1	Y	3	Y	4	**91**	Y	5	F	1	Y	1	F	11			
88	C	0	C	0	C	0	C	0	C	0	C	0	C	0	**92**	C	0	C	0	C	0	C	0			
															93	N	1	A	0	A	0	A	0			
															94	A	0	R	6	R	4	R	17			

FIG. 3C

FRAMEWORK 4 RELATIVE SOLVENT ACCESSIBILITY																					
	VARIABLE KAPPA										V. LAMBDA					VARIABLE HEAVY								
	mouse 12E8		mouse 6FAB		mouse 2FBJ		mouse 1A2Y		rabbit B1mdl		human 2FB4		human 8FAB				mouse 12E8		mouse 6FAB		mouse 2FBJ		rabbit B1mdl	
98	F	7	F	7	F	9	F	8	F	5	F	4	F	8	**103**	W	3	W	10	W	11	W	9
99	G	1	G	1	G	3	G	2	G	2	G	1	G	2	**104**	G	0	G	5	G	1	G	1
100	A	27	G	**30**	A	**47**	G	**36**	G	26	T	30	G	**33**	**105**	Q	17	Q	**36**	Q	**45**	Q	**32**
101	G	8	G	4	G	6	G	7	G	13	G	6	G	7	**106**	G	6	G	11	G	14	G	10
102	T	1	T	0	T	0	T	1	T	0	T	0	T	0	**107**	T	0	T	1	T	8	V	8
103	K	24	K	27	K	26	K	**32**	E	16	K	**33**	K	18	**108**	L	29	T	29	L	12	L	**35**
104	L	2	L	2	L	1	L	3	V	15	V	0	L	0	**109**	V	0	L	1	V	0	V	0
105	E	8	E	5	E	12	E	25	V	21	T	19	T	4	**110**	T	11	T	13	T	8	T	20
106	L	19	I	5	L	4	I	**49**	V	**46**	V	4	V	6	**111**	V	4	V	12	V	5	V	2
107	K	29	K	27	K	**35**	K	**45**	K	**45**	L	30	L	16	**112**	S	10	S	10	S	12	S	23
															113	A	**40**	S	**38**	A	**35**	S	**48**

FIG. 3D

```
VH SEQUENCES
Hu Human target sequence: Germline  VH3-66 + JH4
St Structure for homology modeling 1IGT chain A

B1 CDRs:                        LSFYMC    CIYSGSSGSTYYASWAKG                    SASSTTFHYFNL
                                 CDR1            CDR2                                CDR3
            1         2         3     4              7         8         9              11
   123456789012345678901234567890 67890123456789 67890123456789012abc345678901234 34567890123
St EVKLQESGGGLVQPGGSLKLSCATSGFTFS WVRQTPEKRLEWVA RFTISRDNAKNTLYLQMSRLKSEDTAMYYCAR WGQGTTVTVSS
Hu EVQLVESGGGLVQPGGSLRLSCAASGFTVS WVRQAPGKGLEWVS RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR WGQGTLVTVSS
B1  QSLEESGGGLVKPGASLALTCKASGFSFS WVRQAPGKGLEWIA RFTISKTSA-TTVTLQMTTLTAADTATYFCAR WGQGTLVTVSS
Ra -QSVEESGGRLVTPGTPLTLTCTVSGFSLS WVRQAPGKGLEWIG RFTISKTS--TTVDLKITSPTTEDTATYFCAR WGTGTLVTISS
Ra -QSVKESEGGLFKPTDTLTLTCTVSGFSLS WVRQAPGNGLEWIG RSTITRNTNLNTVTLKMTSLTAADTATYFCAR WGQGTLVTVSS
Ra -QSLEESGGDLVKPGASLTLTCTASGFSFS WVRQAPGKGLEWIA RFTISKTSS-TTVTLQMTSLTAADTATYFCAR WGPGTLVTVSS
Hu EVQLLESGGGLVQPGGSLRLSCAASGFTFS WVRQAPGKGLEWVS RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK WGRGTLVTVSS
Hu EVQLVETGGGLIQPGGSLRLSCAASGFTVS WVRQAPGKGLEWVS RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR WGQGTMVTVSS
Hu EVQLVESGGGLVQPGGSLRLSCAASGFTVS WVRQAPGKGLEWVS RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR WGQGTTVTVSS
Mo QVQLKESGPGLVAPSQSLSITCTVSGFSLT WVRQPPGKGLEWLG RLSISKDNSKSQVFLKMNSLQTDDTAMYYCAR WGQGTLVTVSA
Mo EVMLVESGGGLVKPGGSLKLSCAASGFTFS WVRQTPEKRLEWVA RFTISRDNAKNNLYLQMSSLRSEDTALYYCAR WGAGTTVTVSS
Mo EVKLVESGGGLVKPGGSLKLSCAASGFTFS WVRQSPEKRLEWVA RFTISRDNAKNTLYLQMSSLKSEDTAMYYCTR WGQGTTLTVSS
      ---  -----  --------       ----- ------- -------      -------         ------- ----------
       A     A'       B            C      C'      D             E                F         G

VK SEQUENCES
St Structure for homology modeling 1IGT chain B
Hu Human target sequence: Germline  VK L12 + JK4
B1 CDRs:               QASDNIYSLLA     YTSDLTS                          QSYHYSKSSTYVNV
                          CDR1           CDR2                                CDR3

            1         2          4          6          7         8         10
   12345678901234567890123 567890123456789 789012345678 901234567890123456 78 8901234567
St DIVLTQSPSSLSASLGDTITITC WYQQKPGNIPKLLIY GVPSRFSGSGSG--TGFTLTISSLQPEDIATYYC FGGGTKLEIK
Hu DIQMTQSPSTLSASVGDRVTITC WYQQKPGKAPKLLIY GVPSRFSGSGSG--TEFTLTISSLQPDDFATYYC FGGGTKVEIK
B1 DIVMTQTPSSVSAAVGGTVTIKC WYQQKPGQPPKLLIY GVPSRFSGSGYG--TEFTLTISDLECADAATYYC FGGGTEVVVK
Ra AYDMTQTPASVEVAVGGTVTIKC WYQQKPGQRPKLLIY GVSSRFKGSGSG--TEFTLTISGVECADAATYYC FGGGTEVVVK
Ra DVVMTQTPASVSEPVGGTVTIKC WYQQKPGQPPKLLIS GVSSRFKASRSG--TEFTLTISDLECADAATYYC FGGGTKVVVE
Ra ALVMTQTPASVSAAVGGTVTIKC WYQQKPGQPPKLLIY GVPSRFKGSRSG--TEYTLTISGVQREDAATYYC FGGGTELEIL
Ra EVVMTQTPASVEAAVGGTVTIKC WYQQKPGQRPNLLIY GVPSRFKGSRSG--TEFTLTISGVQREDAATYYC FGTGTKVEIK
Hu DIQMTQSPSSLSASVGDRVTITC WYQQKPGKAPKLLIY GVPSRFSGSGSG--TDFTLTISSLQPEDFATYYC FGQGTKLEIK
Hu DIQMTQSPSSVSASVGDRVTITC WYQQKPGKAPKLLIY GVPSRFSGSGSG--TDFTLTISSLQPEDFATYYC FGPGTKVDIK
Hu AIQMTQSPSSLSASVGDRVTITC WYQQKPGKAPKLLIY GVPSRFSGSGSG--TDFTLTISSLQPEDFATYYC FGGGTKVEIK
Hu EIVMTQSPATLSVSPGERATLSC WYQQKPGQAPRLLIY GIPARFSGSGSG--TEFTLTISSLQSEDFAVYYC FGQGTRLEIK
Mo DIQMNQSPSSLSASLGDTITITC WYQQKKGNIPKLLIY GVPSRFSGSGSG--TGFTLTISSLQPEDIATYYC FGGGTKLEIK
Mo DIVMTQSPSSLSVSAGDKVTMSC WYQQKPWQPPKLLIY GVPDRFTGSGSG--TDFTLTISSVQAEDLAVYYC FGSGTKLEIK
Mo DIQMTQSPASLSASVGETVTITC WYQQKQGKSPQLLVY GVPSRFSGSGSG--TQYSLKINSLQPEDFGSYYC FSDGTRLEIK
Mo SIVMTQTPKFLPVSAGDRVTMTC WYQQKPGQSPKLLIY GVPDRFTGSGSG--TDFTFTISSVQVEDLAVYFC FGAGTKLELK
      ---    -----   ------ -----  -------      -------     -------         ----- ---------
       A      A'      B      C        C'            D            E               F       G
```

Fig. 4